

From the Dermatological Clinic of the University of Zurich

Director: Prof. Dr. G. Miescher

**A Study by Means of Paper Electrophoresis of the Changes
in the Protein Components of Guinea Pig Serum during the
Development of Contact Eczema to Dinitrochlorbenzoni**

INAUGURAL-DISSERTATION

FOR THE

DEGREE OF DOCTOR OF MEDICINE

FROM THE

MEDICAL FACULTY OF

THE

UNIVERSITY OF ZURICH

PRESENTED BY

HELEN GEDULDIG-STAPP

OF NEW YORK

ACCEPTED ON THE PROPOSAL OF
PROF. DR. G. MIESCHER

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INTRODUCTION

The allergic basis of eczema has been almost universally accepted. The causative agents are numerous including those due to drugs, parasites, micro-organisms, and exogenic agents. The latter comprise the large group of contact eczema, the recognized etiology of which concerns a specific hypersensitivity of the skin to various substances when applied epicutaneously. The antigen-antibody reaction involved in this condition, and more specifically the means of transmission of the same, has been a topic of extensive discussion for many years. The specificity of the reaction concerned, as well as the existence of a latent period between exposure to the antigen and appearance of the sensitivity are typical occurrences in the antigen-antibody reaction.

The fact that contact eczema may be caused by relatively simple compounds such as nickel and mercury salts, resorcin, etc., does not contradict the antigen-antibody process. LANDSTEINER (1) showed that a variety of simple chemical compounds including 2,4 dinitrochlorbenzol (DNCB) acquire antigenic properties by linkage to proteins to form a complex antigen. The antibody produced after injection of such an antigen can react with the simple compound in a specific manner.

However the failure to conclusively demonstrate the presence of antibodies in the blood of the sensitized animal as well as the inability to obtain a positive PRAUSNITZ-KUSTNER phenomenon question the existence of an antigen-antibody reaction (2, 3).

Recently HAXTHAUSEN (4) has reported that he has been able to passively transmit allergic eczema by means of injecting lymphocytes from the sensitized to the nonsensitized animal. The role of the lymphocyte as the possible transmitter of the antibody in contact eczema is a noteworthy development.

The means of transfer of the allergen from a localized area to other parts of the skin is still not known. SIMON (3), LANDSTEINER and CHASE (5), KALKOFF (6), and HAXTHAUSEN (7) have shown in their experiments that an intact skin is not necessary for the transfer of the allergen to other parts of the skin. They report that the lymph and blood streams possibly play a role in its transmission. Works by MIESCHER (8), SCHNITZER (9), and STORCK (10) express doubt that the transmission of the allergen or complex-allergen occurs by means of the epi-

dermis, lymph or blood streams from the locally sensitized area. These authors indicate that the vegetative nervous system plays an important role. However, conclusive evidence indicating the means of transfer of the allergen awaits further study.

Recently much work has been done on the electrophoretic analysis of serum in patients with dermatoses including contact eczema in order to further investigate the mechanism involved in skin allergy (11, 12, 13, 14, 15). Some of these results show definite protein changes from the normal. It has been observed by LEIN-BROCK (13) that the α globulin is increased in cases of acute inflammatory dermatoses and that the γ globulin is increased in chronic skin disorders. LEVER (12) reported an increase in the γ globulin in cases of extensive eczema. These results indicate that the allergic process may be responsible for the changes in the serum protein fractions. However the possibility remains that the changes are secondary due to toxins, loss of fluid and proteins in extensive vesiculation or to super-infection. It should also be remarked that almost all of these studies were performed on cases of extensive and chronic eczema with few exceptions on localized and acute conditions.

In order to further investigate the changes which occur in the serum in cases of contact eczema as reported above, it was decided that a systematic study of the serum protein fractions by means of paper electrophoresis throughout the sensitization and development of an acute localized contact eczema to DNCB on guinea pigs should be done. Although the protein fractions of normal guinea pig serum show great variations from each other, it was nevertheless decided to use these animals instead of rabbits which show more stable values. The advantage in employing the guinea pig as the laboratory animal lies in the ease with which it may be sensitized. It may also be assumed that by using a large number of animals a mean may be obtained which would minimize the wide variations observed.

Since the introduction of the electrophoretic separation of proteins by TISELIUS (16), it has become one of the most widely used methods to study protein fractions. WIELAND and FISCHER (17), TURBA and ENENKEL (18) and CREMER and TISELIUS (19) have introduced a more convenient method known as paper electrophoresis. GRASSMANN (20, 21) claims that his modification of paper electrophoresis compares favorably with results obtained by the use of the TISELIUS apparatus.

The electrophoretic study, using the TISELIUS apparatus, of circulating antibodies in conditions where they can be observed, such as in immune sera, was done by TISELIUS and KABAT (22), MOORE et al (23) and NEWELL et al (24). Their results show that the antibodies wander with the γ globulin component of the serum. LOVELESS and CANN (25) and CAMPBELL et al (26), on the other

hand, report antibody activity to be associated with the α and β as well as the γ globulin in some cases. The method of electrophoresis convection was used to fractionate the serum components in the latter studies.

Because of the facility of the paper electrophoresis method and the reliability claimed by its proponents, it was chosen as the method of study of protein fractions to be employed in this work.

THE TECHNIC OF PAPER ELECTROPHORESIS

After GRASSMANN (20, 21) using the Elphor apparatus of HOBEIN & BENDER

A light pencil line about 2 cm. long is drawn 10 cm. from the left end of Whatman filter paper no. 1 (4 x 40 cm.) perpendicular to the long axis of the paper leaving a free margin of about 0.5 cm. on either side of the pencil mark. The paper is then saturated with the sodium veronal-sodium acetate buffer, pH 8.6 with an ionic strength of 0.1, and suspended on the carrier rack. 0.01 ccm. of serum is applied over the pencil mark by means of a graduated pipette. The rack is placed in a chamber containing the buffer and then covered to produce a saturated atmosphere. Direct current of 110 volts is placed across the chamber with the serum closer to the negative pole allowing it to wander toward the positive pole. After running for 8 hours, the paper strips are removed and dried in an oven at about 100° C for 10 minutes. They are then stained with amidoblack 10-B for 10 minutes, after which they are destained in a solution of glacial acetic acid in methanol (1:9) until the non-protein containing part of the paper is a light pale blue color. The paper is dipped into a mixture of α -bromnaphtaline and paraffine oil to make it translucent. It is then read in a photometer and the results are recorded on millimeter graph paper. The electrophoretic pattern thus obtained is subjectively interpreted into Gaussian curves of the albumin, α , β and γ globulin fractions. The respective areas are then determined with a planimeter and the values of albumin, α , β and γ globulines are calculated in relative percent.

EXPERIMENTAL WORK

Young guinea pigs weighing from 300 to 400 grams were selected. In the first experiment, animals of both sexes were used. However because of possible changes in the serum protein fractions due to pregnancy only males were chosen in the subsequent experiment. Blood was taken from 7 normal animals at repeated intervals throughout the experiment.

A 5 % DNCB solution in alcohol was applied epicutaneously to the epilated sacral region of a total of 42 guinea pigs covering an area about 4 cm. in diameter. During the sensitization period of 12 days, 1 ccm. of heart blood was taken daily from 3 animals at a time. The typical cutaneous changes due to DNCB were observed. At first a marked reddening is apparent which diminishes in the course of 2 to 3 days. Infiltration of the area becomes evident a day after the application and gradually a crust develops. Scaling begins in 3 to 4 days and is completed by the 8th to 9th day of sensitization.

The development of contact eczema was exhibited by the epicutaneous application of 1/4 % DNCB to another epilated portion of back skin. In the first group of 12 animals, an area of 4 cm. in diameter was painted 12 days after the application with 5 % DNCB. In the second group consisting of 30 animals, contact eczema was developed at two different times, respectively 20 and 40 days after sensitization with 5 % DNCB. At first a skin area of 4 cm. in diameter was painted on half of the animals, while the others had half of their backs painted. Later application of 1/4 % DNCB was made again on an area of 4 cm. in diameter in half the animals and on the entire back in the remainder. This was done because changes in the protein fractions were reported in cases of extensive dermatitis (12, 13).

Animals which had never been sensitized with 5 % DNCB did not show any sign of development of a contact eczema when 1/4 % DNCB was applied to their epilated backs.

During the development of the contact eczema, blood was taken twice daily, again from 3 animals at a time, until the scaling was completed, then once daily for a week and finally every other day for another week. In the second group of 30 animals, 6 animals were sensitized but never painted with 1/4 % DNCB. Blood

from these animals was taken along with the others and served as a comparison with both the hypersensitive and normal animals. The cutaneous changes during the development of hypersensitivity were similar to those observed during the sensitization.

Since guinea pig serum hemolyses very readily, a record of the degree of hemolysis was kept to determine whether it had any influence upon the electrophoretic results. It was found to have no bearing on the results.

As has been reported by MOORE (27), a clear separation of the β and γ globulin fractions is very difficult to obtain in guinea pig serum. In an attempt to achieve a clearer separation, the electrophoresis system was allowed to run for varying lengths of time. A series of strips was run for 24 hours. However the diffusion was so great that it was impossible to interpret the electrophoretic pattern into Gaussian curves and therefore the protein fractions could not be determined. It was found that the best separation occurred after 8 to 9 hours, although a sharp distinction between β and γ globulines are rarely obtained.

DISCUSSION OF RESULTS

The results are summarized in Table 1. An obvious change in the various protein components compared to the normal was not observed. An attempt was therefore made to analyse the results statistically. This was done by determining the standard deviations of results during the experiment and of the normal animals.

Since blood was taken from 3 to 6 animals each day, it was necessary to arrange the results into larger groups in order to be able to consider them statistically. The grouping was done as follows:

A. Normal animals

B. Control animals (i.e. those animals which were sensitized but never given 1/4 % DNCB to develop an eczema.)

C. During the sensitization with 5 % DNCB

1. before scaling
2. during scaling
3. after scaling

D. During the development of contact eczema with 1/4 % DNCB

1. before scaling
 - a. on an area of 4 cm. in diameter

Experimental results:

Mean values and their standard deviations expressed in relative percent

Table 1

	No. of animals	No. of curves	Albumin	α	Globulins β	γ
A. normals	7	60	50,6 4,4	26,6 4,0	8,3 1,8	14,4 3,4
B. Controls	6	50	49,6 3,8	22,6 4,8	8,3 2,4	14,5 3,9
C. Sensitization	42					
1. before scaling		32	52,1 $\pm$ 5,8	25,7 $\pm$ 4,3	8,9 $\pm$ 2,7	13,3 $\pm$ 4,8
2. during scaling		40	49,0 $\pm$ 5,0	26,7 $\pm$ 3,9	9,1 $\pm$ 2,4	15,2 $\pm$ 4,4
3. after scaling		12	47,8 $\pm$ 5,8	29,9 $\pm$ 3,4	9,2 $\pm$ 1,8	13,1 $\pm$ 2,0
D. Hypersensitivity						
1) before scaling						
a. 4 cm area	23	47	47,8 $\pm$ 3,9	25,9 $\pm$ 2,3	10,0 $\pm$ 2,5	16,3 $\pm$ 4,1
b. 1/2 back	8	10	50,6 $\pm$ 2,4	23,7 $\pm$ 3,6	10,1 $\pm$ 1,9	15,6 $\pm$ 2,9
c. entire back	12	14	45,7 $\pm$ 2,7	27,5 $\pm$ 5,4	10,4 $\pm$ 2,7	16,4 $\pm$ 4,0
2) during scaling						
a. 4 cm area	23	65	48,8 $\pm$ 5,7	27,8 $\pm$ 3,8	8,3 $\pm$ 1,4	15,1 $\pm$ 4,3
b. 1/2 back	8	12	49,9 $\pm$ 4,8	27,2 $\pm$ 5,2	8,5 $\pm$ 2,2	14,5 $\pm$ 3,5
c. entire back	12	28	48,6 $\pm$ 3,6	29,2 $\pm$ 3,7	8,8 $\pm$ 1,9	13,4 $\pm$ 3,3
3) after scaling						
a. 4 cm area	23	41	49,3 $\pm$ 4,7	26,9 $\pm$ 3,0	8,7 $\pm$ 1,4	15,1 $\pm$ 4,7
b. 1/2 back	8	6	52,5 $\pm$ 1,0	28,0 $\pm$ 3,9	7,2 $\pm$ 1,4	12,3 $\pm$ 4,4
c. entire back	12	11	51,9 $\pm$ 4,7	25,9 $\pm$ 5,9	8,0 $\pm$ 3,5	14,3 $\pm$ 5,7

- b. on half the back
- c. on the entire back
- 2. during scaling
 - a. on an area of 4 cm. in diameter
 - b. on half the back
 - c. on the entire back
- 3. after scaling
 - a. on an area of 4 cm. in diameter
 - b. on half the back
 - c. on the entire back

Standard deviation was determined by the formula:

$$\sigma = \sqrt{\frac{\sum (x - \bar{x})^2}{n}} \quad \text{where}$$

x = individual result

$\bar{x}$ = the mean

n = the number of individual results.

As can be seen from Table 1, no marked changes are apparent. To determine whether those changes which exist are significant, the following formula was used:

$$t = \frac{\bar{x}_1 - \bar{x}_2}{\sqrt{\frac{\sum (x_1 - \bar{x}_1)^2}{n_1} + \frac{\sum (x_2 - \bar{x}_2)^2}{n_2}}} \sqrt{\frac{n_1 n_2}{n_1 + n_2}} \quad \text{where}$$

t is an arbitrary value to determine whether a significant difference exists between two groups of results.

From the "t" values obtained it was seen that no significant differences were present.

These negative results can be explained in one of two ways. The first is that no quantitative changes occur in the serum protein fractions during the sensitization and development of contact eczema in guinea pigs. This would be in agreement with the fact that passive transfer to contact eczema has never been successful with serum (2, 3).

The second explanation of the negative results lies in the method of paper electrophoresis. ESSER et al (28), GRASSMANN and HANNIG (21), RIVA and MARTIN (29), OTT et al (30), FLYNN (31) and WUNDERLY et al (32) have shown that paper electrophoresis is adequate for clinical determinations. However it has not been found to be as accurate as the TISELIUS method according to RIVA

and MARTIN (29). OTT (30) reported the greatest difference to occur in the β globulin. Because the method of paper electrophoresis has a larger experimental error than the TISELIUS method, only relatively large deviations from the norm can be considered significant. The two methods give discordant results especially when there is only a small deviation of the pathological curve from the normal.

Aside from this, authors (27, 33, 34) who have worked with the TISELIUS method on guinea pig serum have not reported uniform results. Table 2 shows a comparison of the normal values for albumin, α , β and γ globulins. As can be seen, these normal values vary considerably from each other. The results not only show a marked difference, one from the other, but also a wide variation in the values obtained in normal guinea pig serum. SVENSSON (33) showed a range from 46.8 to 64.4 relative percent in the albumin fraction, whereas MOORE had a range from 42 to 66 relative percent. The author's own values for albumin ran from 41.6 to 60.0 relative percent in normal animals. In addition EDSALL (35) states that most of the electrophoretic components of plasma are far from homogenous. Cholesterol, phosphatids and fatty acids are closely bound to the α and β globulins. Aside from that, ultra centrifugation shows that the γ globulin consists of faster and slower sedimentation, that is, it is not uniform.

The question whether changes in the serum protein fractions occur during the sensitization and hypersensitivity to DNCB has not been satisfactorily answered. If changes occur they lay within the range of experimental error of this method and await a more accurate means of determination.

A comparison of the mean values expressed in relative percent of electrophoretic studies on serum and plasma of normal guinea pigs

Table 2

Author	Method	No. of animals	Globulins				Fibrinogen
			Albumin	α	β	γ	
SVENSSON (13)	TISELIUS	--	55, 8	14, 5	8, 2	21, 5	--
MOORE (14)	TISELIUS	15	54	32	6	8	--
DEUTSCH & GOODLOE (15)	TISELIUS	3	54, 6	22, 9	8, 8	5, 6	8, 1
GEDULDIG (1)	paper electrophoresis	7	50, 6 $\pm$ 4, 4	26, 6 $\pm$ 4, 0	8, 4 $\pm$ 1, 8	14, 4 $\pm$ 3, 4	--
Own results	paper electrophoresis	17	47, 8 $\pm$ 3, 5	27, 1 $\pm$ 1, 2	8, 5 $\pm$ 1, 4	16, 6 $\pm$ 3, 5	--

SUMMARY

An attempt was made to determine whether changes in the serum protein fractions occurred in experimental contact eczema. Heart blood from a total of 42 guinea pigs was taken daily during the sensitization with 5 % DNCB and during the development of hypersensitivity with 1/4 % DNCB. The sera were then studied by means of paper electrophoresis. No apparent changes in the serum protein fractions could be demonstrated by the use of this method (paper electrophoresis after GRASSMANN).

There are two possibilities explaining the negative results.

1. No quantitative changes occur in the serum protein fractions in experimental contact eczema, which results would be in agreement with the fact that passive transfer of eczema has never been successful using serum.

2. If changes exist they are within the limits of experimental error of this paper, and are due either

- a. to the wide variation obtained in normal guinea pig serum, or,
- b. to this method of paper electrophoresis which may not be sensitive enough to record small changes.

REFERENCES

1. Landsteiner, K.: Die Spezifität der serologischen Reaktionen, Berlin 1933
2. Landsteiner, K. and Jacobs, J.: J. of Exp. Med. 61, 643 (1935)
3. Simon, F.A.: J. of Immun. 30, 275 (1936)
4. Haxthausen, H.: Acta Derm. - Vererol. 31, 659 (1951)
5. Landsteiner, K. and Chase, M.W.: J. of Exp. Med. 69, 767 (1939)
6. Kalkoff, K.W.: Arch. für Derm. u. Syph. 186, 493 (1948)
7. Haxthausen, H.: Acta Derm. - Venerol. 20, 257 (1939)
8. Miescher, G.: Schw. Med. Wschr. 71, 15 (1941)
9. Schnitzer, A.: Dermatologica 88, 306 (1943)
10. Storck, H.: Int'l. Arch. of Allergy of Appl. Immun. Suppl. ad Vol. 1, 25 (1950)
11. Robert, P.: Dermatologica 97, 89 (1948)
12. Lever, W.F.: Schultz, E.L., Hurley, N.A.: A.M.A. Arch. of Derm. and Syph. 63, 6 (1951)
13. Leinbrock, A.: Hautarzt 3, 9 (1952)
14. Rockl, H., Jaroschka, R.: Hautarzt 3 (june 1952)
15. Antweiler, J.: Die quantitative Elektrophorese in der Medizin, Springer Verlag 1952 (section by Leinbrock, A. 138-180)
16. Tiselius, A.: Nova Acta Reg. Soc. Sci. Upsaliensis 7, ser. IV, no. 4 (1930)
17. Wieland, Th., Fischer, E.: Naturwiss. 35, 29 (1948)
18. Turba, F. and Enenkel, A.J.: Naturwiss. 37, 93 (1950)
19. Cremer, H.D. and Tiselius, A.: Biochem. Z. 320, 273 (1950)
20. Grassmann, W., Hannig, K., Knedel, M.: Dtsch. Med. Wschr. 76, 333 (1951)
21. Grassmann, W. and Hannig, K.: Hoppe-Seyler's Ztschr. für Physiolog. Chemie 290, 1 (1952)
22. Tiselius, A. and Kabat, E.A.: J. of Exp. Med. 69, 119 (1939)
23. Moore, D.H., van der Scheer, J., Wyckoff, W.G.: J. of Immun. 38, 221 (1940)

24. Newell, J.M., Sterling, A., Oxman, M.F., Burden, S.S., Krejci, L.E.:
J. of Allergy 10, 513 (1939)
25. Loveless, M.H. and Cann, J.R.: Science 117, 105 (1953)
26. Campbell, D.H., Cann, J.R., Friedman, T.B., Brown, R.A.: J. of Allergy
21, 519 (1950)
27. Moore, D.H.: J. of Biol. Chem. 161, 21 (1945)
28. Esser, H., Heinzler, Fr., Kazmeier, F., Scholtan, W.: Münch. Med.
Wschr. 93, 985 (1951)
29. Riva, G. and Martini, V.: Schw. Med. Wschr. 83, 5 (1953)
30. Ott, H., Huber, H., Korner, G.: Klin. Wschr. 30, 34 (1952)
31. Flynn, F.V., de Mayo, P.: Lancet 261, 235 (1951)
32. Wunderly, Ch., Schneider, G., Hugentobler, F.: Comptes rendus du troisi-
ème Congres de la Société Internationale Euro-
péenne d'Hématologie (1951)
33. Svensson, H.: Ark. for Kemi, Miner. och Geol. 22A, 135
(1946)
34. Deutsch, H.F. and Goodloe, M.B.: J. of Biol. Chem. 161, 1 (1945)
35. Edsall, J.: Adv. in Prot. Chem. 3, 383 (1947)

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CURRICULUM VITAE

On the 9th of July 1927, I was born in the City of New York, where I attended primary and secondary schools. From 1944 to 1948 I was enrolled in Hunter College of the City of New York, from which I received the degree of Bachelor of Arts. I worked as a laboratory technician from January to September 1948. In August 1948 I was married to Roy Geduldig.

In the fall of 1948 I matriculated into the medical faculty of the University of Zurich. After attending all the semesters at Zurich I passed the medical "Staatsexamen" for foreigners in the fall of 1953.

